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# Identification of Three Lineages of Wild Measles Virus by Nucleotide Sequence Analysis of N, P, M, F, and L Genes in Japan

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The nucleotide sequences of nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), and large protein (L) genes were partly determined for 19 wild strains of measles virus (MV) isolated over the past 10 years in Japan (nucleotide position N: 1301–1700, P: 1751–2190, M: 3571–4057, F: 6621–7210, L: 10381–11133) and also for a MV strain obtained from a patient with subacute sclerosing panencephalitis (SSPE) who had natural measles in 1980. The phylogenetic trees of these strains drawn for respective genes were very similar to each other and revealed that all the wild strains were classified chronologically into 3 subgroups, those isolated in 1984, 1984–1989, and 1990–1994. The SSPE strain was classified into the subgroup of 1984. Phylogenetic tree analyses including other strains in the world revealed that Japanese strains in 1984 were classified into a distinct lineage which might correlate with the European strains from late 1970s to mid 1980s. Japanese strains from 1984 to 1989 were almost identical to those of the United States isolated from 1989 to 1992, and Japanese strains in 1990s were related closely to some of the MV strains isolated in 1994 in the United States. Genetic recombination among the MV genes seemed not to have occurred. ***J. Med. Virol. 52:113–120, 1997.***



**KEY WORDS:** wild strains; classification; chronological change; phylogenetic tree; SSPE

## INTRODUCTION

Measles virus infection is responsible for approximately 1 million deaths in developing countries each year. The expanded programme on immunization (EPI) is in progress and is expected to eradicate measles from the world [Keja et al., 1988], although many problems make this goal difficult to achieve. In developing countries, one of the major difficulties is how to immunize infants who have maternal antibody against measles. For this purpose, a synthetic peptide vaccine is thought to be a candidate, and some laboratories have attempted to identify the B or T cell recognition epitopes of MV in order to develop an immunogenic peptide vaccine for measles [Beauverger et al., 1994; Verjans et al., 1995; Hummel and Bellini, 1995; Makela et al., 1989; Obeid et al., 1995].

Taylor et al. [1991] described some different lineages of wild strains of MV co-circulating at a time, and Rota et al. [1992, 1994b] reported that significant antigenic drift was observed in the H protein of MV and that recent isolates of MV were classified into significantly different lineages from those isolated previously. Therefore it is important to analyze as many strains as possible to detect the genetic changes of MV.

We reported previously on the sequences of part of the N and H genes of wild MV strains in Japan [Nakayama et al., 1995]. According to those sequences, it was possible to classify chronologically the wild strains in Japan into three subgroups: strains of 1984, of 1985–1989, and of 1990–1993. The H protein is an envelope protein, and it is possible that the genetic changes in the H region would be influenced by antibody pressure. But it was unknown whether the major genetic changes had occurred in the other genes such as P, M, F, and L, as well as in the H gene. The nucleotide sequences of part of the P, M, F, and L genes and the more extended region of the N gene were examined of various strains. We also analyzed the same regions for a strain of SSPE and compared it with the wild strains.

## MATERIALS AND METHODS

### Patients and Measles Strains

All the wild strains of measles virus were isolated in Kanto district from 1984 to 1994. The year of isolation, specimen, and used cell lines are listed in Table I.

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TABLE I. Measles Virus Strains Reported and Referred to in this Study

| Strain | Accession no. N | P | M | F | L | Year | Material | Cell line |
|---|---|---|---|---|---|---|---|---|
| 84-K*# | D87053 | D87751 | D87562 | D87582 | D87602 | 1984 | PBMC | Vero |
| 84-E*# | D87483 | D87522 | D87563 | D87583 | D87603 | 1984 | PBMC | Vero |
| 84-S* | D87475 | D87523 | D87564 | D87584 | D87604 | 1984 | PBMC | Vero |
| 84-Y*# | D87482 | D87524 | D87565 | D87585 | D87605 | 1984 | NPS | Vero |
| 84-I*# | D87481 | D87525 | D87566 | D87586 | D87606 | 1984 | NPS | Vero |
| 87-K# | D87476 | D87527 | D87567 | D87587 | D87607 | 1987 | NPS | B95a |
| 89-T# | D87485 | D87526 | D87568 | D87588 | D87608 | 1989 | NPS | B95a |
| 89-H*# | D87490 | D87528 | D87569 | D87589 | D87609 | 1989 | NPS | Vero |
| 89-U* | D87486 | D87529 | D87570 | D87590 | D87610 | 1989 | PBMC | B95a |
| 90-O*# | D87488 | D87530 | D87571 | D87591 | D87611 | 1990 | NPS | Vero |
| 90-K# | D87474 | D87531 | D87572 | D87592 | D87612 | 1990 | NPS | B95a |
| 90-OK | D87489 | D87532 | D87573 | D87593 | D87613 | 1990 | NPS | B95a |
| 91-T* | D87480 | D87533 | D87574 | D87594 | D87614 | 1991 | NPS | B95a |
| 91-O | D87477 | D87534 | D87575 | D87595 | D87615 | 1991 | PBMC | B95a |
| 92-S | D87484 | D87535 | D87576 | D87596 | D87616 | 1992 | PBMC | B95a |
| 92-E | D87487 | D87536 | D87577 | D87597 | D87617 | 1992 | NPS | B95a |
| 93-KA# | D87479 | D87537 | D87578 | D87598 | D87618 | 1993 | PBMC | B95a |
| 94-H | D87478 | D87538 | D87579 | D87599 | D87619 | 1994 | PBMC | B95a |
| 94-E | D87473 | D87539 | D87580 | D87600 | D87620 | 1994 | PBMC | B95a |
| SSPE93 | D87472 | D87540 | D87581 | D87601 | D87621 | 1993 | Brain | not done |
| AIK-C | S58435 | S58435 | S58435 | S58435 | S58435 | Edm-derived vaccine | | |
| CAM | U03650 | | | | | Tanabe-derived vaccine/Japan/1968 | | |
| Phi26 | U01991 | L36045 | U01986 | U08416 | | Wild/USA/1957 | | |
| Nagahata | D63927 | D63927 | D63925 | D63924 | D63924 | Wild/Japan/1971 | | |
| Pt.A | X16567 | X16567 | X16567 | X16567 | | SSPE/Germany/mid 80s | | |
| Sma94 | X84865 | | | | | SSPE/Spain/1994/1968 | | |
| SBI | X84874 | | | | | Wild/Germany/1992 | | |
| JM | D01002 | M90469 | U01983 | | D37774 | Wild/USA/1977 | | |
| Pt.B | X16568 | X16568 | X16568 | X16568 | | SSPE/Austria/mid 80s | | |
| TN 1994 | L46760 | | | | | Wild/USA/1994 | | |
| DL | X84873 | | | | D37773 | Wild/Germany/1992 | | |
| Ma94B | X84863 | | | | | Wild/Spain/1994 | | |
| NJ 1994 | L46750 | | | | | Wild/USA/1994 | | |
| MVO | D01004 | | | | | Wild/UK/1974 | | |
| SD | U01995 | | U01997 | M81901 | | Wild/USA/1989 | | |
| TX 1992 | L46764 | | | | | Wild/USA/1992 | | |
| Chi 1 | U01977 | | U01980 | M81903 | | Wild/USA/1989 | | |
| WA 1994 | L46767 | | | | | Wild/USA/1994 | | |
| Case C | X16569 | X16569 | X16569 | X16569 | | MIBE/USA/late 70s | | |
| CM | D01003 | M89920 | | | D37772 | Wild/USA/late 70s | | |
| BER83 | U01974 | | U01975 | | | Wild/USA/1983 | | |
| BOS83 | U01990 | | U01985 | | | Wild/USA/1983 | | |
| Gambia91 | L46733 | | | | | Wild/Gambia/1991 | | |
| Y14 | U01998 | | | | | Wild/Cameroon/1983 | | |
| R113 | U01993 | | | | | Wild/Gabon/1984 | | |
| Udem | | | | | M20865 | Edm adapted to HeLa cells | | |
| Yamagata | | | D12682 | | | SSPE/Japan/mid 80s | | |
| Halonen | | | U01982 | | | Wild/Finland/1962 | | |

The nucleotide sequences of the N and H regions have been reported for *125bp and for #546bp, respectively [Nakayama et al., 1995]
PBMC: peripheral blood mononuclear cells; Edm: Edmonston strain; NPS: nasopharyngeal secretions.

Strains 84-K to 94-E were propagated in Vero or B95a cell lines within a passage history of 5 times. The nucleotide sequences of some of the strains for the N and H genes had been reported [Nakayama et al., 1995]. The 84-I and 84-Y strains were isolated in 1984 by Dr. Kobune, Department of Virology, National Institutes of Health, Japan, and were kindly donated to us. Brain specimen from a patient with SSPE was generously provided by Dr. Matsuzaka of Nagasaki University in 1993. This patient was a fourteen-year-old girl who was diagnosed with SSPE in 1993 and had a past history of natural measles in 1980.

### RNA Extraction, Primer Design, and RT-PCR

Total RNA was extracted from 200 ul of infected culture media of the wild strains and from the SSPE brain specimen using guanidinium thiocyanate as reported by Chomczynski and Sacchi [1987]. We synthesized the sets of linker-primers for each region, referring to the sequence of AIK-C strain [Mori et al., 1993]. The genomic locations of the primers are illustrated in Figure 1. Measles genomic RNA was first converted to cDNA with NPB0 positive sense primer for the N and P regions, MP3 primer for the M region, MF5 primer for the F region, and MF1 primer for the L region. The proce-

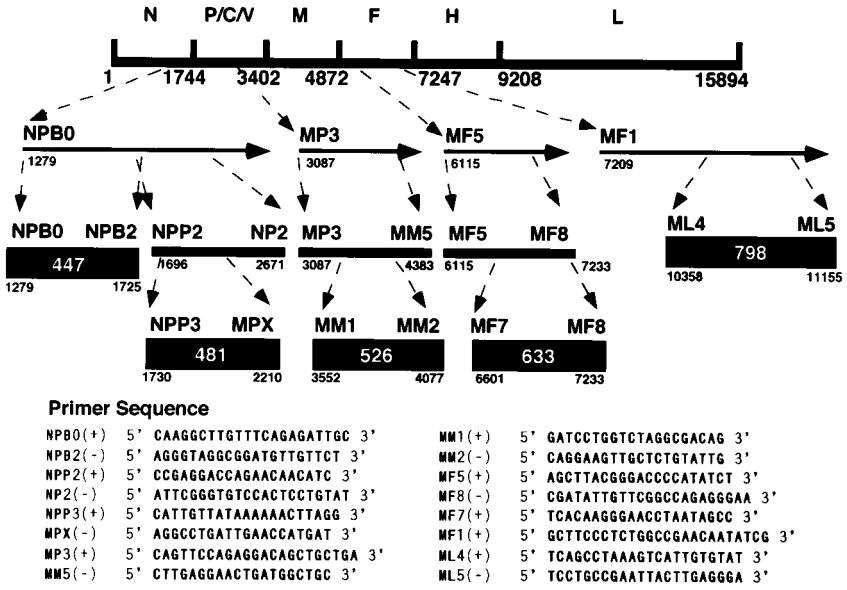


Fig. 1. Primers used in this study. The extracted RNA was reverse-transcribed to cDNA with NPB0, MP3, MF5, and MF1 primers. Each cDNA was used as a template for single or nested PCR for respective genes. The number of nucleotide position is based on the genomic location of the AIK-C strain.

dures of reverse-transcription and polymerase chain reaction (PCR) have been described previously [Nakayama et al., 1995].

The 447 bp of DNA fragment of the N region was amplified with a set of NPB0(+) and NPB2(−) primers. As for the P region, the first PCR was done with a set of NPP2(+) and NP2(−) primers, amplifying the 976 bp of DNA. Then we amplified the 481 bp of DNA by nested PCR with a set of NPP3(+) and MPX(−) primers. As for the M region, the first PCR was done with a set of MP3(+) and MM5(−) primers, amplifying the 1297 bp of DNA. The 526 bp of DNA was then amplified by nested PCR with MM1(+) and MM2(−) primers. The F region was amplified for the 1119 bp of DNA by the first PCR with MF5(+) and MF8(−) primers. Then semi-nested PCR was done with a set of MF7(+) and MF8(−) primers, amplifying the 633 bp of the DNA. The L region was prepared using ML4(+) and ML5(−) primers with or without the −21M13 universal sequence at the 5′-end, and was amplified for the 798 bp of DNA fragment with a set of −21M13-ML4(+) and ML5(−) primers or a set of ML4(+) and −21M13-ML5(−) primers.

### Nucleotide Sequence of the PCR Product

Fifty ul of the PCR product was electrophoresed through low-melting-temperature 1% agarose gel and the specific DNA band was excised from the gel. For the N, P, M, and F regions, the PCR products were digested with the specific linker restriction enzymes and were inserted into pUC118 or pUC119 plasmid vectors which had been digested with the appropriate restriction enzymes. PCR was carried out subsequently for each of the ligated vectors with the M13 universal sequencing primer and the opposite site specific primer of the passenger DNA. PCR products were purified and the DNA sequences were analyzed bi-directionally with an automated nucleotide analyzer, 373A DNA sequencer (Applied Biosystems, Foster City, CA). The L region was analyzed without ligation procedure. The number of the nucleotide location is based on the sequence of AIK-C vaccine strain [Mori et al., 1993]. Primer sequences are omitted in the reported nucleotide sequences.

### Nucleotide Sequence Analyses

The nucleotide sequence data reported in this paper have been deposited in the DDBJ database under accession numbers shown in the upper column of Table I. The nucleotide alignments and phylogenetic analyses were done with the GENETYX-MAC version 8.0 program (Software Development Co., Ltd.). All the phylogenetic trees were drawn by the neighbor-joining algorithm as unrooted trees without any parameter changes of the program.

## RESULTS

### Phylogenetic Analyses of Japanese Strains for Each Gene

Figure 2 shows a phylogenetic tree of 20 MV strains analyzed in this report drawn for the N gene (nucleotide position 1301–1700). This region is well known for its genetic diversity [Rima et al., 1995a, 1995b; Taylor et al., 1991; Rota et al., 1994b], and is used for a "signature" sequence for specific genotypes. All the strains were clearly classified into 3 groups, A, B, and C,

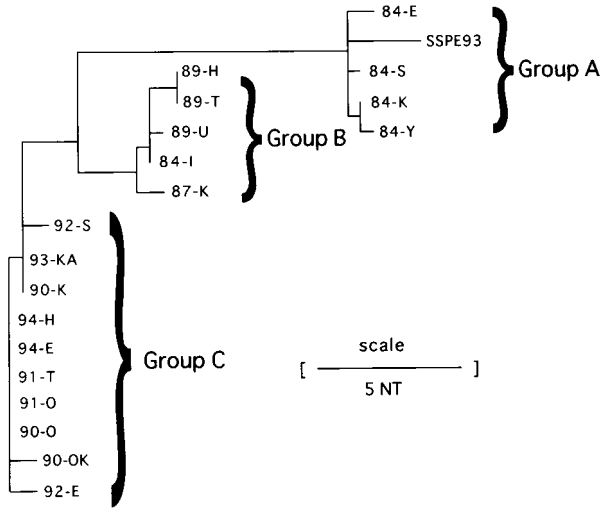


Fig. 2. Phylogenetic tree of Japanese strains for the N gene. Unrooted phylogenetic tree drawn for the C-terminal 400 bp of the N gene. Nineteen Japanese wild strains isolated from 1984 to 1994 were chronologically classified into 3 groups, A, B, and C. SSPE93 strain, the origin of which seemed to be in 1980, belonged to Group A.

chronologically. The SSPE93 strain was derived from the brain of a patient with SSPE who had had measles in 1980 and had been diagnosed with SSPE in 1993. This strain was also classified in Group A, which consisted of 4 strains isolated in 1984. Group B consisted of 5 strains which were isolated from 1984 to 1989, and Group C included 10 strains isolated from 1990 to 1994.

The phylogenetic trees of these strains were examined for the other genes, P, M, F, and L (data not shown). All the trees were very similar to each other, and the same classification as the N gene was possible for every gene. The SSPE93 strain was also classified into Group A for these genes.

### Phylogenetic Analyses With Other Strains

In order to construct phylogenetic trees including other strains in the world, the sequences from each group were combined into a single consensus sequence for respective genes. These consensus sequences are shown in Figure 3 with the result of SSPE93 strain. Table II shows the number of nucleotide differences between the individual sequence and the consensus. Each sequence varies from the consensus by $\leqq$1.0% except for that of 84-Y strain in Group A for the M, F, and L genes.

The nucleotide sequences of various MV strains for each gene were taken from the GenBank database, and the accession numbers, together with the origin of the viruses, are shown in Table I.

Figure 4a shows the phylogenetic tree drawn for the N gene (nucleotide position 1301–1685). The Japanese Group A consensus belonged to the cluster which included Nagahata and SSPE93 strains. The Nagahata strain is a wild MV isolated in Japan in 1971 [Wong et al., 1991]. The SSPE93 strain was derived directly from brain tissue of the patient who had had primary measles infection in 1980, and the close relationship between Group A and SSPE93 indicates the persistence of the original measles virus in the brain. Japanese Group B (1984–1989) shared an ancestral node with the cluster of SD, TX 1992, and Chi1 strains [Rota et al., 1994b, 1996]. These strains were isolated in the United States from 1989 to 1992, when the resurgence of measles cases was reported. On the other hand, Japanese Group C (1990–1994) belonged to the same cluster with WA 1994 strain which was isolated in the United States in 1994 [Rota et al., 1996].

Figure 4b shows the phylogenetic tree drawn for the P gene (nucleotide position 1767–2190). There were only limited numbers of MV strains available for this region in the database, and the shape of this tree was essentially the same as that reported by Baczko et al. [1992]. The relationship among Japanese Group A, SSPE93, and Pt. A strain in the P gene was similar to that of the N gene, but there was a discrepancy—that the Nagahata strain, which was related closely to the group A in the N gene, belonged to another cluster in the P gene. Groups B and C shared a close ancestral node, and they belonged to another cluster.

Figure 4c shows the phylogenetic tree of the M gene (nucleotide position 3571–4057). The region analyzed to construct this tree occupied about half the length of the entire coding region of the M gene. This tree included the Yamagata and Case C strains, which were SSPE and measles inclusion body encephalitis (MIBE) viruses, respectively [Cattaneo et al., 1989]. As reported by Cattaneo et al. [1986], these persistent viruses had many mutations in the M gene, and they were located very far from the common node in the tree, which caused compression of the horizontal scale of this tree as compared to the other genes. The relationship among the other strains were essentially the same as that of the N gene, except for that of the Pt. A strain, which shared the same node with the cluster of the Japanese Group A, the SSPE93, and the Nagahata strains in the N gene, belonged to another branch sharing the node with AIK-C and other old strains in late 1950s to 1960s.

Figure 4d shows the phylogenetic tree drawn for the F gene (nucleotide position 6621–7110). This tree was based on the nucleotide sequences of the C-terminus of the F gene coding region. The relationship between the strains was also similar to that of the M gene, in which the Pt. A strain belonged to another branch from the Japanese old strains. Japanese Group B was also closely related to the SD and Chi1 strains in the F gene, and Group C shared the same ancestral node with this cluster. SSPE93 strain had a point mutation of C to T at nucleotide 7036 (Fig. 3), which generated another stop codon in the cytoplasmic domain of fusion protein. Due to this mutation, the deduced amino acid sequence of SSPE93 strain was truncated by 24 residues (data

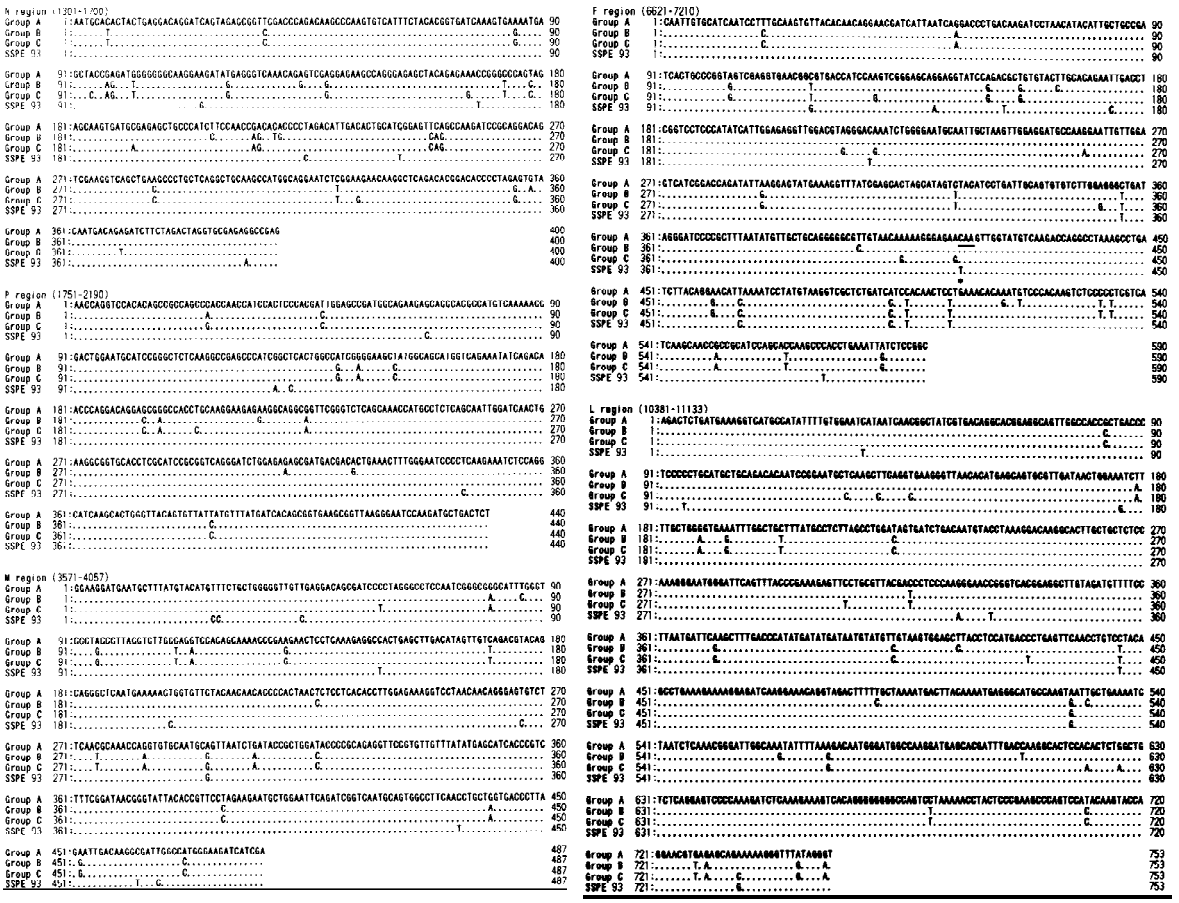

Fig. 3. Nucleotide alignment of the consensus sequences and SSPE93 strain "*" and an underline in the F region shows the point mutation of SSPE93 strain which generated another stop codon in the cytoplasmic domain of the F gene.

TABLE II. Nucleotide Difference With the Consensus Sequence

| Strain | N/400 | P/440 | M/487 | F/590 | L/753 bp |
|---|---|---|---|---|---|
| 84-K | 1 | 1 | 5 | 0 | 2 |
| 84-E | 2 | 1 | 2 | 2 | 1 |
| 84-S | 1 | 1 | 1 | 1 | 4 |
| 84-Y | 2 | 1 | 10 | 9 | 7 |
| 84-I | 0 | 0 | 0 | 1 | 0 |
| 87-K | 3 | 1 | 1 | 2 | 0 |
| 89-T | 2 | 1 | 1 | 0 | 1 |
| 89-H | 2 | 0 | 2 | 1 | 2 |
| 89-U | 1 | 1 | 0 | 2 | 2 |
| 90-O | 0 | 2 | 0 | 3 | 0 |
| 90-K | 1 | 1 | 0 | 3 | 0 |
| 90-OK | 2 | 0 | 1 | 3 | 0 |
| 91-T | 0 | 1 | 4 | 1 | 4 |
| 91-O | 0 | 1 | 3 | 2 | 0 |
| 92-S | 3 | 2 | 1 | 1 | 0 |
| 92-E | 2 | 1 | 2 | 1 | 1 |
| 93-KA | 1 | 1 | 1 | 0 | 4 |
| 94-H | 0 | 0 | 4 | 1 | 4 |
| 94-E | 0 | 0 | 5 | 1 | 4 |

not shown) as other SSPE strains reported previously [Schmid et al., 1992; Billeter et al., 1994].

There were only a few sequence data of MV L gene in the database. Figure 4e shows the phylogenetic tree of L gene based on currently available sequences (nucleotide position 10381–11133). The relationship between the Nagahata, the SSPE93, and the Japanese Group A was the same as other genes. The DL and JM strains belonged to the same cluster in the L gene which coincided with the N gene.

## DISCUSSION

We have already reported that wild strains of MV in Japan were classified chronologically into 3 subgroups by the analysis of H gene nucleotide sequences [Nakayama et al., 1995]. Sakata et al. [1993] have also described that Japanese MV strains were classified similarly into 3 groups by SDS-PAGE analysis of the H protein. In this study, we extended the number of wild strains analyzed and confirmed further that this classification was observed equally in the other genes of Japanese MV strains. Table II shows that each consensus sequence coincides well with the individual sequences, which means that this classification is appropriate. Only the 84-Y strain had significant nucleotide differences from the consensus for the M, F, and L genes. Although it is not certain why this discrepancy occurred, we feel confident that this classification of 84-Y in Group A is permissible, considering the nucleotide coincidence seen in the N and P genes.

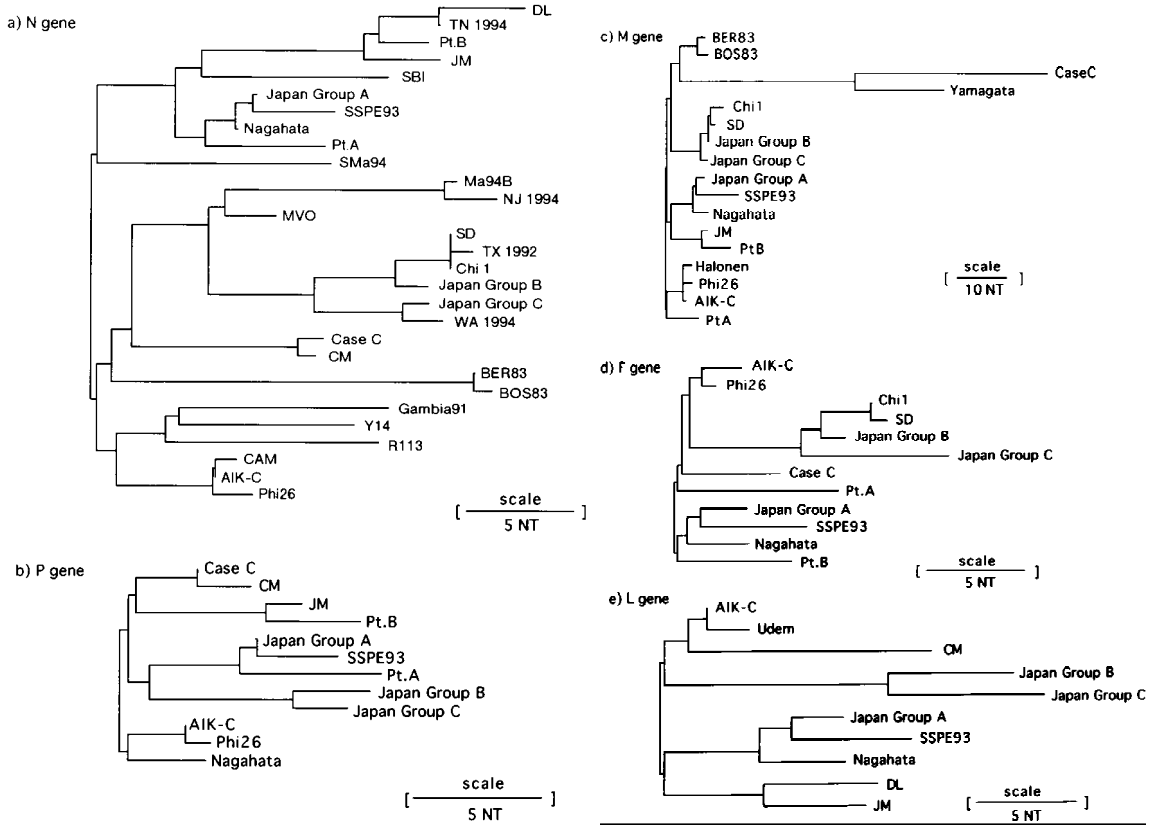


Fig. 4. Phylogenetic trees drawn for each gene. Each tree was drawn by a neighbor-joining algorithm. Scales for these unrooted trees are based on number of nucleotide changes. Strain abbreviations and origins are as described in Table I.

Several studies have described the divergence of the C-terminus of the MV N gene and constructed phylogenetic trees based on the nucleotide sequences of this region [Taylor et al., 1991; Rota et al., 1994a, 1994b, 1995, 1996; Rima et al., 1995a, 1995b]. The overall structure of Figure 4a is basically the same as those reported previously. Our results show that the Japanese group A was in close relation with the Nagahata strain. It is curious that wild MV strains in 1971 were almost identical to those isolated in 1984 in Japan. As shown in Figure 2, the SSPE93 strain, whose origin was in 1980, was also closely related to Group A. These findings mean that wild MV strains of this genotype have been distributed widely in Japan from the 1970s to the early 1980s. This cluster shared an ancestral node with the Pt. A strain, which was derived from the SSPE case of Germany in the mid 1980s [Cattaneo et al., 1989]. Rima et al. [1995b] classified 65 MV strains into 8 genotypes by phylogenetic analysis of the C-terminus of the N gene and reported that the Pt. A strain belonged to the group C1, which included some SSPE strains in Europe, such as MF, SMa81, and S(K) strains and two wild strains in Spain, Mad78 and Mad79. These strains were isolated in Europe from the early 1970s to the mid 1980s. We think there might have been some relationship between the Japanese Group A and these European strains, considering the coincidence of the period of isolation, but it seems that the Japanese group A is a distinct lineage because the relationship between the group A and the Pt. A strains in the M, F, and L genes were not so closely related as the N and P genes (Fig. 4b–e).

Rota et al. [1996] reported that wild type MV strains isolated in the United States between 1989 and 1992 belonged to a single genotype, which caused the resurgence of measles cases. The Japanese Group B was in close relation with this genotype such as the SD, Chi1, and TX 1992 strains in the N, M, and F genes (Fig. 4a, c, d). Rima et al. [1995b] designated this genotype as subgroup D2 and described that first isolations of the D2 group were made in the United Kingdom in 1988. We found that 84-I strain in the Japanese Group B was earlier than those British strains in this genotype. Whether the D2 group originated in Japan or not is uncertain because of the lack of sequence data of other strains in the world.

The Japanese Group C seemed to correlate with Group B (Fig. 4b, c), although it was clear that both groups were distinct from each other (Fig. 2). Figure 4a shows that Group C was closely related to the WA 1994 strain. Rota et al. [1996] reported that the WA 1994 strain, which was isolated in Seattle, Washington, in 1994, was imported by a Japanese tourist. They reported the phylogenetic trees based on the H and N genes of MV and raised the question of why the Japan/88 strain, which was isolated in Japan in 1988 [Saito et

al., 1994] and belonged to the same lineage as the TX 1992 strain, was not so closely related to the WA 1994 strain. Our results that major lineage of wild MV in Japan had changed in 1990 would explain this.

Baczko et al. [1991, 1992] reported that recombination had not taken place between the N, P, and M genes in some MV strains by comparing the phylogenetic trees of each gene. Rota et al. [1996] reported that phylogenetic trees of the N and H genes of wild MV strains were very similar to each other. In this paper, we described the phylogenetic analyses of the N, P, M, F, and L genes of measles viruses. The overall structure of each tree was basically the same as each other, and these findings would confirm further that recombination of genes among several lineages of MV had not taken place.

As shown in Figures 2 and 3, the Japanese wild type MV was classified into 3 groups chronologically, and this observation means the appearance of a different lineage of MV strains in 1984 and in 1990 in Japan. Interestingly, there were epidemics of measles cases in 1984 and in 1991 in Japan [Official Surveillance of Infectious Diseases, Japan], but it is not certain whether these epidemics were directly related to the appearance of new lineages of MV. Tamin et al. [1994] reported that one or several neutralization epitopes were conserved between the old MV such as the Edmonston strain and recent wild strains, while there were some new or modified epitopes on the recent MV strains, such as the Chi1 strain, which were not present on the vaccine strain. They noted that the contribution of antigenic changes to the epidemiology of MV infections was unclear, but it might be possible that the antigenic changes, when combined with waning immunity, were sufficient to increase infection and transmission within highly vaccinated populations. We consider that our finding that the major lineage of wild MV strains have changed sequentially in Japan might be related to this suggestion.

Rota et al. [1996] reported that most measles cases in the United States during 1994 were thought to have been imported from other countries. It is essential to know the chronological molecular epidemiology of MV strains in each country in order to clarify the relationship of MV lineages in the world.

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## REFERENCES

Baczko K, Brinckmann U, Pardowitz I, Rima BK, ter-Meulen V (1991): Nucleotide sequence of the genes encoding the matrix protein of two wild type measles virus strains. Journal of General Virology 72:2279–2282.

Baczko K, Pardowitz I, Rima BK, ter-Meulen V (1992): Constant and variable regions of measles virus proteins encoded by the nucleocapsid and phosphoprotein genes derived from lytic and persistent viruses. Virology 190:469–474.

Beauverger P, Buckland R, Wild F (1994): Measles virus hemagglutinin induces an Ld-restricted CD8+ cytotoxic T lymphocyte response to two specific epitopes. Virology 200:281–283.

Billeter MA, Cattaneo R, Spielhofer P, Kaelin K, Huber M, Schmid A, Baczko K, Ter-Meulen V (1994): Generation and properties of measles virus mutations typically associated with subacute sclerosing panencephalitis. Slow Infections of the Central Nervous System 724:367–377.

Cattaneo R, Schmid A, Rebmann G, Baczko K, Ter-Meulen V, Bellini WJ, Rozenblatt S, Billeter MA (1986): Accumulated measles virus mutations in a case of subacute sclerosing panencephalitis: Interrupted matrix protein reading frame and transcription alteration. Virology 154:97–107.

Cattaneo R, Schmid A, Spielhofer P, Kaelin K, Baczko K, ter-Meulen V, Pardowitz J, Flanagan S, Rima BK, Udem SA, Billeter MA (1989): Mutated and hypermutated genes of persistent measles viruses which caused lethal human brain diseases. Virology 173: 415–425.

Chomczynski P, Sacchi N (1987): Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry 162:156–159.

Hummel KB, Bellini WJ (1995): Localization of monoclonal antibody epitopes and functional domains in the hemagglutinin protein of measles virus. Journal of Virology 69:1913–1916.

Keja K, Chan C, Hayden G, Henderson RH (1988): Expanded programme on immunization. World Health Statistics Quarterly 41: 59–63.

Makela MJ, Lund GA, Salmi AA (1989): Antigenicity of the measles virus haemagglutinin studied by using synthetic peptides. Journal of General Virology 70:603–614.

Mori T, Sasaki K, Hashimoto H, Makino S (1993): Molecular cloning and complete nucleotide sequence of genomic RNA of the AIK-C strain of attenuated measles virus. Virus Genes 7:67–81.

Nakayama T, Mori T, Yamaguchi S, Sonoda S, Asamura S, Yamashita R, Takeuchi Y, Urano T (1995): Detection of measles virus genome directly from clinical samples by reverse transcriptase-polymerase chain reaction and genetic variability. Virus Research 35:1–16.

Obeid OE, Partidos CD, Howard CR, Steward MW (1995): Protection against morbillivirus-induced encephalitis by immunization with a rationally designed synthetic peptide vaccine containing B- and T-cell epitopes from the fusion protein of measles virus. Journal of Virology 69:1420–1428.

Rima BK, Earle JAP, Baczko K, Rota PA, Bellini WJ (1995a): Measles virus strain variations. Current Topics in Microbiology and Immunology 191:65–83.

Rima BK, Earle JAP, Yeo RP, Herlihy L, Baczko K, ter-Meulen V, Carabana J, Caballero M, Celma ML, Munoz-Fernandez R (1995b): Temporal and geographical distribution of measles virus genotypes. Journal of General Virology 76:1173–1180.

Rota JS, Hummel KB, Rota PA, Bellini WJ (1992): Genetic variability of the glycoprotein genes of current wild-type measles isolates. Virology 188:135–142.

Rota JS, Wang ZD, Rota PA, Bellini WJ (1994a): Comparison of sequences of the H, F, and N coding genes of measles virus vaccine strains. Virus Research 31:317–330.

Rota PA, Bloom AE, Vanchiere JA, Bellini WJ (1994b): Evolution of the nucleoprotein and matrix genes of wild-type strains of measles virus isolated from recent epidemics. Virology 198:724–730.

Rota PA, Rota JS, Bellini WJ (1995): Molecular epidemiology of measles virus. Seminars in Virology 6:379–386.

Rota JS, Heath JL, Rota PA, King GE, Celma ML, Carabana J, Fernandez-Munoz R, Brown D, Jin L, Bellini WJ (1996): Molecular epidemiology of measles virus: Identification of pathways of transmission and implications for measles elimination. The Journal of Infectious Diseases 173:32–37.

Saito H, Sato H, Abe M, Harata S, Amano K, Suto T, Morita M (1994): Cloning and characterization of the cDNA encoding the HA protein of a hemagglutination-defective measles virus strain. Virus Genes 8:107–113.

Sakata H, Kobune F, Sato TA, Tanabayashi K, Yamada A, Sugiura A (1993): Variation in field isolates of measles virus during an 8-year period in Japan. Microbiology and Immunology 37:233–237.

Schmid A, Spielhofer P, Cattaneo R, Baczko K, ter-Meulen V, Billeter

MA (1992): Subacute sclerosing panencephalitis is typically characterized by alterations in the fusion protein cytoplasmic domain of the persisting measles virus. Virology 188:910–915.

Tamin A, Rota PA, Wang Z-de, Heath JL, Anderson LJ, Bellini WJ (1994): Antigenic analysis of current wild type and vaccine strains of measles virus. The Journal of Infectious Diseases 170:795–801.

Taylor MJ, Godfrey E, Baczko K, ter-Meulen V, Wild TF, Rima BK (1991): Identification of several different lineages of measles virus. Journal of General Virology 72:83–88.

Verjans GM, Janssen R, UytdeHaag FG, van-Doornik CE, Tommassen J (1995): Intracellular processing and presentation of T cell epitopes, expressed by recombinant *Escherichia coli* and *Salmonella typhimurium,* to human T cells. European Journal of Immunology 25:405–410.

Wong TC, Ayata M, Ueda S, Hirano A (1991): Role of biased hypermutation in evolution of subacute sclerosing panencephalitis virus from progenitor acute measles virus. Journal of Virology 65:2191–2199.